

The present invention further provides methods of treating cystic fibrosis or alleviating the symptoms of cystic fibrosis by administering an agent that decreases or inhibits the activity of UDP glucose:glycoprotein glycosyl transferase.

5 The present invention also provides methods of treating cystic fibrosis or alleviating the symptoms of cystic fibrosis by administering an agent that decreases or inhibits activity of the endoplasmic reticulum Ca^{++} ATPase.

The present invention further provides methods of treating cystic fibrosis or alleviating the symptoms of cystic fibrosis by administering an agent that lowers the concentration of Ca^{++} in the endoplasmic reticulum.

10 The present invention further provides methods of treating cystic fibrosis or alleviating the symptoms of cystic fibrosis by administering an agent that decreases or inhibits calnexin functional activity.

The present invention provides methods of screening candidate compounds to identify an agent that inhibits endoplasmic reticulum-associated retention or degradation of
15 a mis-assembled or mis-folded glycoprotein, wherein the method includes the steps of:

- a) treating a cell exhibiting intracellular retention of a mis-assembled or mis-folded glycoprotein in the endoplasmic reticulum with the candidate compound; and
- b) determining whether the mis-assembled or mis-folded glycoprotein is released from the endoplasmic reticulum, thereby identifying the candidate compound as
20 an agent that causes the release of a malformed mis-folded glycoprotein from the endoplasmic reticulum.

The present invention also provides methods of screening candidate compounds to identify an agent that inhibits the functional activity of UDP glucose:glycoprotein glycosyl transferase, wherein the method includes the steps of:

- 25 a) treating a cell exhibiting intracellular retention of a mis-assembled or mis-folded glycoprotein in the endoplasmic reticulum with the candidate compound; and
- b) determining whether the mis-assembled or mis-folded glycoprotein is released from the endoplasmic reticulum, thereby identifying the candidate compound as an agent that causes the release of a mis-assembled or mis-folded glycoprotein from the
30 endoplasmic reticulum.

The present invention provides aerosol formulations of thapsigargin, DBHQ or cyclopiazonic acid.

In addition, the present invention provides compositions which include two or more of the following agents: 1) an agent that decreases or inhibits the activity of UDP

glucose:glycoprotein glycosyl transferase, 2) an agent that decreases or inhibits activity of the endoplasmic reticulum Ca^{++} ATPase, 3) an agent that increases or stimulates IP_3 receptor activity, 4) an agent that increases or stimulates ryanodine receptor activity, and 5) an agent that decreases or inhibits calnexin functional activity.

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DESCRIPTION OF THE DRAWING

Figure 1. CFTR chloride channel activity in excised patches from CF-affected airway epithelial cells in control conditions or after treatment with thapsigargin. Cells were pretreated with IBMX (100 μM) and forskolin (10 μM) prior to patch excision. Initially patches were held at -50mV, and then stepped through a voltage protocol from +10mV to +90mV. 1mM ATP was present in the bath to prevent channel rundown.

A. Representative single channel current traces from a membrane patch excised from untreated IB3-1 cells. No low conductance chloride channel activity was seen. Arrows indicate closed state.

15 B. Representative single channel currents from a membrane patch excised from an IB3-1 cell after treatment with thapsigargin. Low conductance chloride channel activity can be seen as the downward deflections in the current traces. Arrows indicate closed state.

Figure 2. Characteristics of CFTR channels in CF-affected airway epithelial cells after thapsigargin treatment.

A. The current versus voltage relationship of the low conductance channels depicted in Figure 1B is plotted. The average single channel conductance was 11.8 pS.

B. All points histogram at +80mV. The area under the first peak represents time spent in the closed state, while the area under the second peak represents time spent in the open state. The calculated open state probability is 0.12.

25 **Figure 3.** The effects of elevation of cytosolic cAMP on short circuit current. Monolayers of CFPAC or T84 cells were exposed to a cAMP-stimulation cocktail of 10 μM forskolin and 100 μM IBMX. The bars indicate the % increase in I_{sc} that is furosemide sensitive detected after treatment with the cAMP stimulation cocktail. The asterisks mark a significant difference between untreated CFPAC cells (n=12) and either the thapsigargin treated CFPAC cells (p= 0.02, *) (n=12) or the T84 cells (p=0.004, **) (n = 12). Error Bars =SEM.

Figure 4. Confocal immunofluorescent localization of the mutant $\Delta F508$ CFTR protein in untreated and thapsigargin-treated CF-PAC cells. Untreated CF-PAC cells or CF-PAC cells which had been treated with thapsigargin were subjected to confocal immunofluorescence labeling using an antibody directed against the CFTR protein.

5 When viewed *en face* (A) or in XZ cross-section (C), the untreated cells revealed a staining pattern consistent with an exclusively intracellular localization of the CFTR protein. No cell surface labeling could be detected. In contrast, thapsigargin-treated cells viewed *en face* (B) or in XZ cross-section (D) reveal bright staining of microvilli at the apical plasma membrane. The intracellular signal is markedly diminished in the treated cells. Thus, thapsigargin treatment induces the relocalization of the $\Delta F508$ mutant CFTR protein from an intracellular compartment to its site of appropriate functional residence at the apical cell surface. The width of the monolayer is 11 μ .

Figure 5. Distribution of the $\Delta F508$ CFTR protein in Σ CFBE290⁻ CF airway epithelial cells exposed to nebulized thapsigargin. Σ CFBE290⁻ airway epithelial cells were grown to confluence on permeable filter supports. Cells were exposed to thapsigargin dissolved in the media bathing their apical surfaces (A,B), to nebulized thapsigargin (E,F) or were not thapsigargin-treated (C,D) and processed for immunofluorescence. Panels A, C and E depict the immunofluorescent staining of the $\Delta F508$ CFTR protein; panels B, D and F depict the basolateral localization of the Na,K-ATPase α -subunit. The $\Delta F508$ CFTR protein can not be detected in untreated cells, but is present to the same extent at the apical surfaces of cells treated with nebulized or dissolved thapsigargin. The width of the monolayer is 9 μ .

Figure 6. Western blot showing presence of mature CFTR in thapsigargin treated but not untreated CFPAC cells.

25 **Figure 7.** Tracing of transnasal electrical potential (NPD) difference in normal and CF mutant mice homozygous for the $\Delta F508$ mutation. The tracing represents the time course of the NPD protocol and the response of NPD readings to perfusion with control Ringer solution, Ringer solution with amiloride, low chloride with amiloride, and the addition of isoproterenol to the low chloride solution. For the wild type group CFgroup, n= 4-6 animals. Legend: open squares = untreated wild type mice; filled squares = thapsigargin-treated wild type mice; open circles = untreated CF mutant mice; filled circles = thapsigargin-treated CF mutant mice.